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ISOLATION OF SMALL CELLS FROM AN EXPONENTIAL GROWING CULTURE OF *ESCHERICHIA COLI* BY CENTRIFUGAL ELUTRIATION

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1. Introduction

To study events leading to cell division, synchronized bacterial cultures are very useful. In this paper we describe a method which to our knowledge has not been used for the separation of bacterial cells, i.e. centrifugal elutriation [1]. In this method the sedimentation of bacteria is counteracted by a continuous flow of medium that passes at a selected rate through the centrifuge chamber. At a particular rate the smallest cells are washed out of the centrifuge chamber, while for the larger cells the effect of sedimentation is stronger than that of elution. The bacteria are introduced in their growth medium into the rotor chamber and isolation of a fraction of small cells is achieved within about 10 min.

2. Materials and Methods

2.1. Culture and medium

The organism used was *Escherichia coli* K-12 (dap⁻, lys⁻) obtained from U. Schwarz, Tübingen. Cells were grown in minimal citrate medium [2] with 0.2% glucose, 20 µg/ml lysine and 20 µg/ml diaminopimelic acid in batch cultures of 100 ml at 37°C. The doubling time was 50 min. At an absorbance of 0.8 to 0.9 at 450 nm 12 ml of the culture was loaded into the elutriation rotor. The concentration of small cells obtained in this way is in the order of 10⁷/ml.

2.2. Centrifugal elutriation

For the use of the modified method of elutriation rapid and reproducible setting of rotor speed is

required. This was achieved by alterations of the electrical circuitry of the centrifuge (Beckman J2). The flow rate was generated by means of hydrostatic pressure. A flow meter was incorporated into the system. This modified method of elutriation was described in detail elsewhere [3]. The hydrostatic pressure was 180 cm and the flow rate was adjusted by means of a clamp. All parts of this equipment except the connecting tubing were cooled with water of 4°C. To prevent the creation of air bubbles during elutriation, the medium was boiled prior to use. Elutriation was monitored by tracing the absorbance of the fractions at 280 nm.

2.3. Evaluation of cell separation

The elutriated cells were collected in a flask placed in an ice bath and samples were taken for agar filtration and electron microscopy according to published procedures [4]. Cell length was measured from electron micrographs projected at a final magnification of about 14 000 X onto a transparent tablet digitizer (Summagraphics, Fairfield, Conn.), which was connected to a Hewlett-Packard calculator (HP 9825A).

Upon incubation of the collected cells at 37°C cell number increase was followed with a Coulter counter. The extent of synchronization was measured from the percentage of newborn cell in the collected fractions as determined with the aid of a computer programme described elsewhere [5].

3. Results and Discussion

3.1. Effect of flow rate

The influence of flow rate was tested at 2 ml/min and 4 ml/min. Elutriation was carried out at 5800 rev./min with two rotor chambers in series and with cells fixed in 0.2% formaldehyde. In all experiments the length distribution of the unfractionated exponentially growing cells was compared with the fraction elutriated first. This fraction is expected to represent the smallest cells. Fig. 1 shows that a fraction enriched in small cells can be obtained and that the resolution is improved at the lower flow rate.

3.2. Number of rotor chambers

A flow rate of 2 ml/min at 5800 rev./min was chosen to study the effect on cell separation by

elutriation with one or with two rotor chambers in series. This was done with unfixed bacteria. In Fig. 2 one can see, that better resolution is obtained with two chambers.

3.3. Rotor speed

Unfixed cells from an exponentially growing culture have been centrifuged at various rotor speeds from 4500 to 5800 rev./min at a flow rate of 2 ml/min and with two rotor chambers. Note that the maximum rotor speed is 6000 rev./min. For reasons of safety we did not exceed 5800 rev./min. The results of separations at 5500 and 5800 rev./min are shown in Fig. 3. From the length distribution of the smallest cells it can be seen that a rotor speed of 5800 rev./min gives better results than a rotor speed of 5500 rev./min.

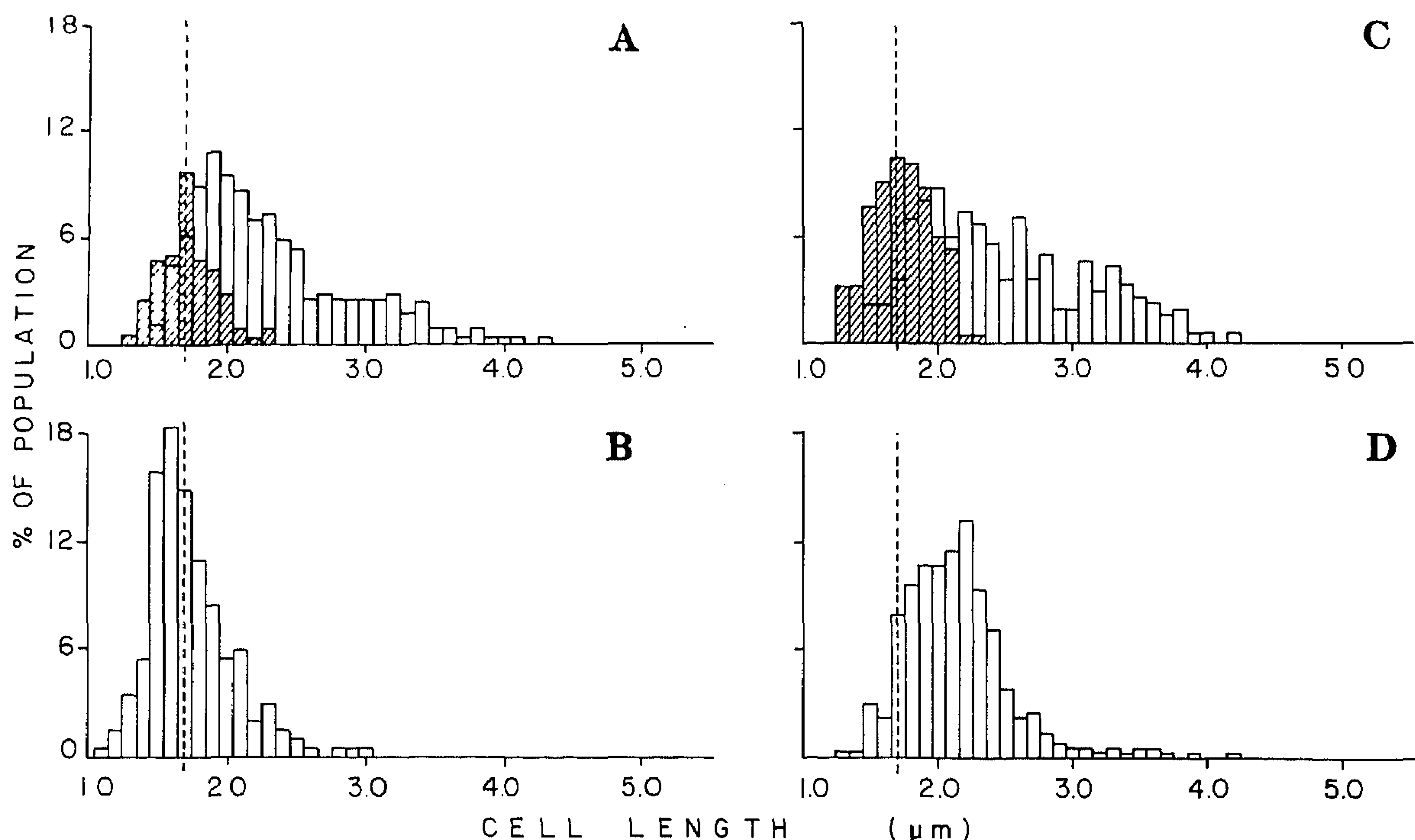


Fig. 1. Separation of formaldehyde-fixed cells at different flow rates. (A) Length distribution of exponentially growing culture (362 cells) with doubling time of 50 min. The hatched area represents the distribution of newborn cells. This distribution was derived from the distribution of the prospective daughters of the dividing cells in the culture. The broken vertical line in all figures represents the median length of the newborn cell. (B) Length distribution of small cells separated at a flow rate of 2 ml/min at 5800 rev./min. (C) Length distribution of exponentially growing culture (294 cells). (D) Length distribution of cells separated with a flow rate of 4 ml/min at 5800 rev./min.

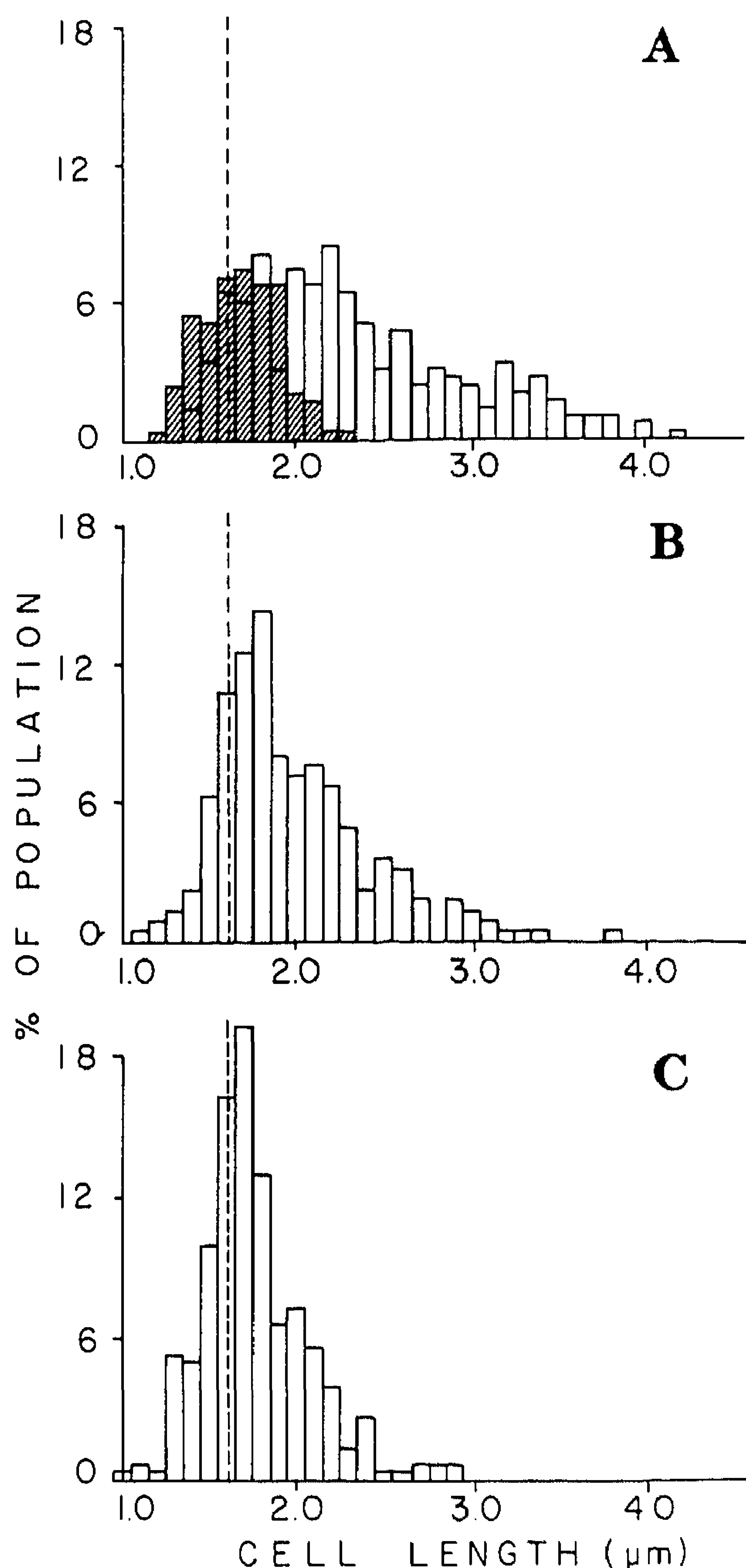


Fig. 2. Separation of unfixed cells with one or two rotor chambers. (A) Length distribution of 295 exponentially growing cells. Hatched area: distribution of newborn cells (cf. Fig. 1A). Broken vertical line: mean length of newborn cells. (B) Length distribution of cells separated with 1 rotor chamber. Flow rate 2 ml/min and rotor speed 5800 rev./min. (C) 2 rotor chambers. Flow rate 2 ml/min and rotor speed 5800 rev./min.

3.4. Synchronization

The smallest cells elutriated at 5500 and 5800 rev./min (Figs. 3C and 3B, respectively) were collected for

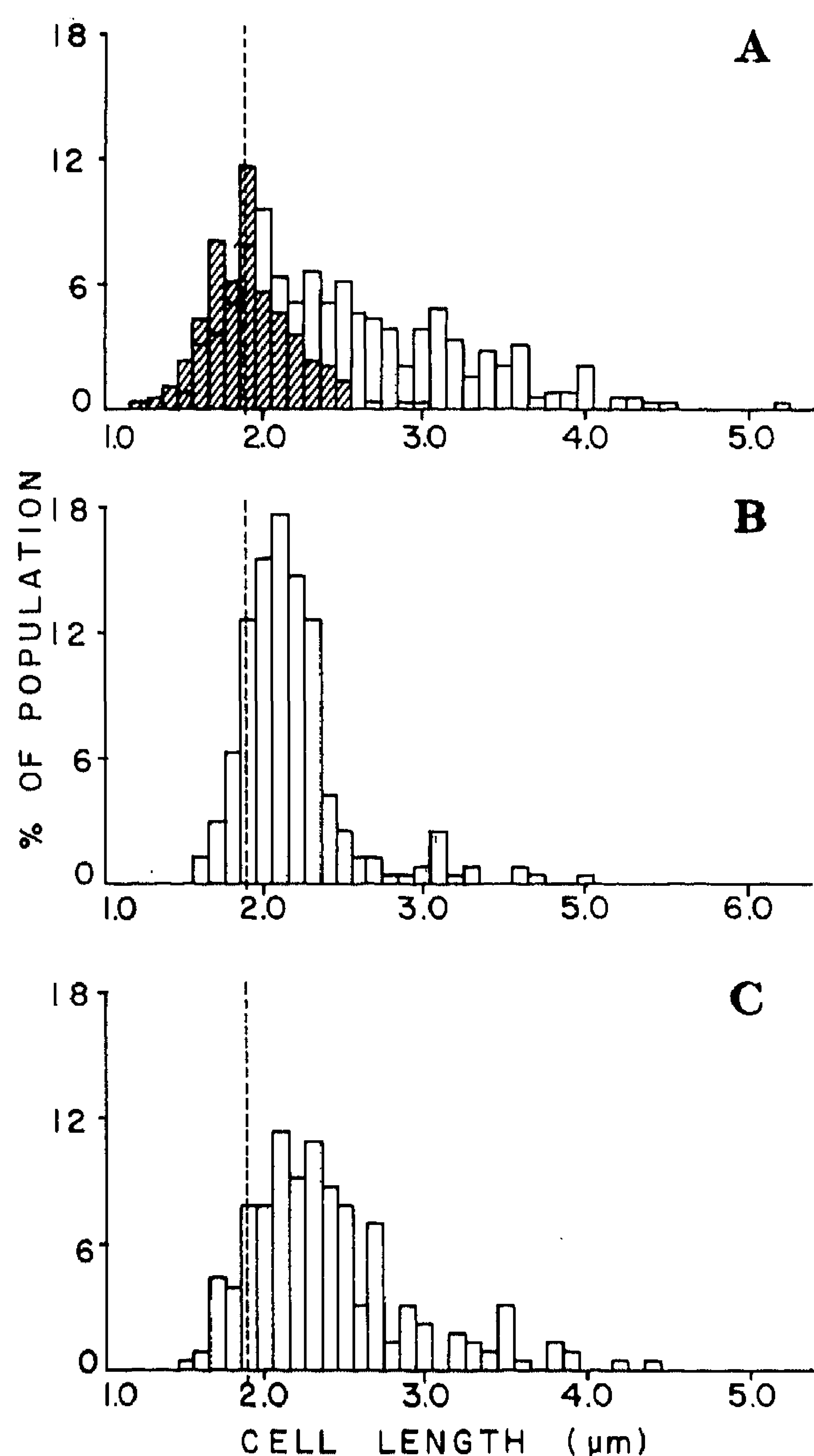


Fig. 3. Separation of unfixed cells at two different rotor speeds. (A) Length distribution of 398 exponentially growing cells. Details as in Fig. 1A. Flow rate 2 ml/min. (B) Cell separation at 5800 rev./min. (C) Cell separation at 5500 rev./min.

11 min, followed by incubation for 2.5 h at 37°C. It can be observed (Fig. 4) that cells separated at 5800 rev./min give a better synchrony than those separated at 5500 rev./min. This is in agreement with the obtained resolutions (cf. length distributions in Figs. 3B and 3C, respectively). This result also indicates that synchrony is not induced by the method, since only cells obtained from elutriation at 5800 rev./min grow synchronously.

To assess the extent of synchronization one has to

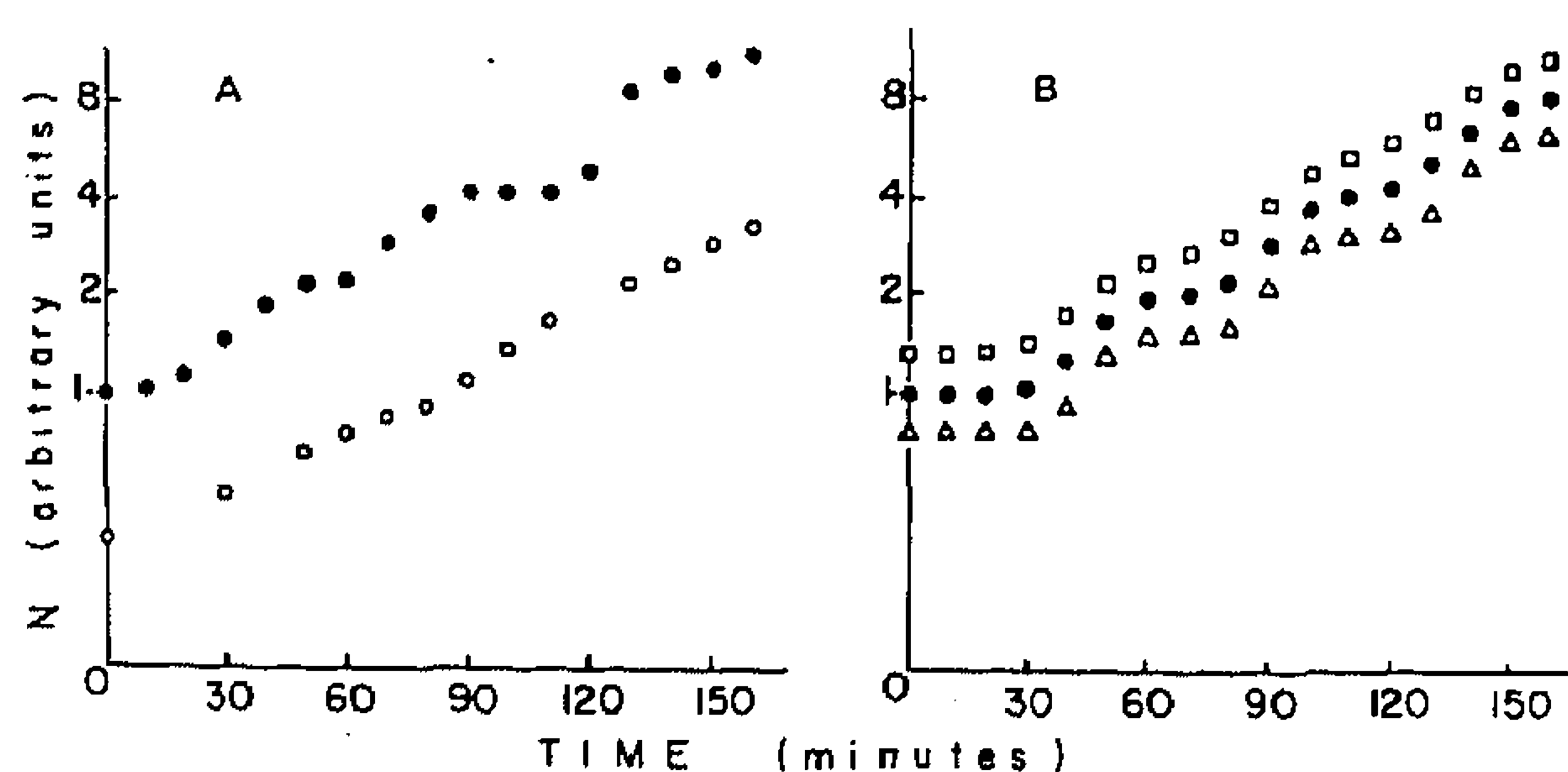


Fig. 4. Experimental and theoretical growth curves of separated cells. (A) The fraction shown in Fig. 3B was resuspended in fresh medium and cell number was followed with a modified Coulter counter (●—●). The fraction of Fig. 3C was dealt with in the same way (○—○). (B) Calculated synchronization curves with coefficients of variation for the interdivision times of 15% (△—△), 20% (●—●), and 25% (□—□), respectively.

take into account the naturally occurring distribution of interdivision times with respect to individual bacteria and the correlation of mother- and daughter cells [6]. The coefficient of variation of the interdivision time can be in the order of 20 to 30% [6–8]. In our experiments we measure the extent of synchronization by an approach in which the “synchronously” growing population is considered as two subpopulations [5]. One fraction (F) contains the actual synchronously growing cells and the other fraction (1-F) represents the contaminating asynchronously growing cells. When applied to a non-synchronised exponential culture, $F = 0$; and in the case of maximal synchronization, $F = 100\%$. The calculated synchronization curves were obtained using three different values for the coefficient of variation of the interdivision times of individual cells, namely 15, 20 and 25%. In all we made the assumption of a correlation coefficient of -0.5 for the interdivision times of mother- and daughter cells [6]. By definition all curves (Fig. 4B) have an F-value of 100. It will be ob-

served that the experimental curve (Fig. 4A) falls in a range between a coefficient of variation of the interdivision of 20 to 25%. The F-value is 90%, which indicates that a degree of synchronization has been achieved close to the maximal one possible.

Centrifugal elutriation may prove to be a useful method for the synchronization of bacteria because the choice of medium is free. Furthermore, it is a rapid method and is, therefore, suitable for cells with relatively short doubling times. Finally, the method is not limited to certain strains as is membrane elution [2].

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